Evolution, 54(5), 2000, pp. 1614–1625

QUANTITATIVE GENETICS OF SEXUAL PLASTICITY: THE ENVIRONMENTAL THRESHOLD MODEL AND GENOTYPE-BY-ENVIRONMENT INTERACTION FOR PHALLUS DEVELOPMENT IN THE SNAIL *BULINUS TRUNCATUS*

MARIE-FRANCE OSTROWSKI,[1] PHILIPPE JARNE, AND PATRICE DAVID
Centre d'Ecologie Fonctionnelle et Evolutive, Centre National de la Recherche Scientifique, 1919 Route de Mende, 34293 Montpellier cedex 5, France
[1]*E-mail: ostrowski@cefe.cnrs-mop.fr*

Abstract.—Sexual polymorphisms are model systems for analyzing the evolution of reproductive strategies. However, their plasticity and other binary traits have rarely been studied, with respect to environmental variables. A possible reason is that, although threshold models offer an adequate quantitative genetics framework for binary traits in a single environment, analyzing their plasticity requires more refined empirical and theoretical approaches. The statistical framework proposed here, based on the environmental threshold model (ETM), should partially fill this gap. This methodology is applied to an empirical dataset on a plastic sexual polymorphism, aphally, in the snail *Bulinus truncatus.* Aphally is characterized by the co-occurrence of regular hermaphrodites (euphallics) together with hermaphrodites deprived of the male copulatory organ (aphallics). Reaction norms were determined for 40 inbred lines, distributed at three temperatures, in a first experiment. A second experiment allowed us to rule out maternal effects. We confirmed the existence of high broad-sense heritabilities as well as a positive effect of high temperatures on aphally. However a significant genotype-by-environment interaction was detected for the first time, suggesting that sexual plasticity itself can respond to selection. A nested series of four ETM-like models was developed for estimating genetical effects on both mean aphally rate and plasticity. These models were tested using a maximum-likelihood procedure and fitted to aphally data. Although no perfect fit of models to data was observed, the refined versions of ETM models conveniently reduce the analysis of complex reaction norms of binary traits into standard quantitative genetics parameters, such as genetic values and environmental variances.

Key words.—*Bulinus truncatus,* environmental determination, inbred lines, phally polymorphism, self-fertilization, threshold traits.

Received October 19, 1999. Accepted March 13, 2000.

Sexual polymorphisms are characterized by the co-occurrence of different sexual morphs within the same population of hermaphroditic species (Darwin 1877). They constitute model systems for analyzing the evolution of mating systems, because sexual morphs represent alternative reproductive strategies (e.g., different selfing rates). Most of the work on sexual polymorphisms has been performed in plants, with gynodioecy being probably the most classical example (for review, see Couvet et al. 1990). However, examples can be found in animals, such as aphally (Larambergue 1939) or androdioecy (Sassaman 1989). A focal point in all studies of sexual polymorphisms is analyzing factors determining sexual morphs. The role of genetic factors has repeatedly been shown (Van Damme 1983; Barrett 1994; Doums et al. 1996a). However, the influence of environmental factors has been considered far less often. For example, a few studies are available in cleistogamous plants (Waller 1980; Paoletti and Holsinger 1999; see also examples of environmental sex determination in animals in Janzen and Paukstis 1991). In snails, various environmental cues strongly influence the sexual morph (Schrag and Read 1992; Baur et al. 1993; Doums et al. 1996a,b). Detailed studies of sexual phenotypic plasticity, that is, the production of different sexual morphs by a single genotype when placed in different environments (Schrag et al. 1994b; Doums et al. 1996a), are therefore of great importance for predicting the evolutionary fate of sexual polymorphisms. Indeed, selection could act not only on morph frequencies, but also on individual phenotypic plasticity (Roff 1986; Doums et al. 1996a).

Aphally is a sexual polymorphism in Pulmonate snails (review in Doums et al. 1998b). It is defined by the co-occurrence of regular hermaphrodites, referred to as euphallic individuals, together with aphallic individuals, which do not have the male copulatory organ. Both sexual morphs can self-fertilize and outcross, although aphallic individuals outcross only as females. Most studies of this trait have been conducted in the freshwater snail *Bulinus truncatus* (Larambergue 1939; Schrag and Read 1992; Doums et al. 1996b). Aphally has a complex genetic basis in this species (Larambergue 1939; Doums and Jarne 1996; Doums et al. 1996b). Temperature also affects the determination of sexual morph, with its increase leading to increased frequencies of aphallic individuals (aphally ratio, or AR), whether in the laboratory (Schrag and Read 1992; Doums et al. 1996a,b, 1998b) or in natural populations (Schrag et al. 1994a,b). Analyzing accurately the determination of sexual polymorphisms such as aphally requires quantitative genetic models.

Binary traits, such as aphally, are usually analyzed under the threshold model (TM; Falconer and Mackay 1996; Roff 1997; Lynch and Walsh 1998). However, the TM does not include environmental effects (e.g., temperature). This led to the development of the environmental threshold model (ETM), what takes phenotypic plasticity into account (Hazel et al. 1990). Briefly, the ETM assumes that genotypes code for an individual threshold, *T,* relative to an environmental variable. Individuals develop into one morph when raised in an environment below *T* and into the other morph when raised above *T.* This phenotypic plasticity is often referred to as ''conditional strategies'' (Hazel et al. 1990). Roff (1994, 1996) suggested analyzing plastic binary traits using the

ETM, and Doums et al. (1996a,b) adapted it for the case of aphally. In the original formulation of the ETM, *T* behaves as a classical quantitative trait following a normal distribution in the population. The original model also admits nongenetic variance of *T* as a source of individual variation (i.e., microenvironmental variance, V_E). However, V_E is assumed a priori to be constant across genotypes, and therefore has no impact on the fate of conditional strategies. Both Roff (1994) and Doums et al. (1996a,b) found this last assumption to be consistent with empirical data. However, these authors did not use the adequate quantitative genetics design for evaluating, or even detecting, V_E. Moreover, the basic assumptions of the ETM have been only partially tested, and no standard procedure is available to measure its goodness of fit to actual data.

The aim of this paper is to estimate the genetic and environmental components of phenotypic variance of aphally in *B. truncatus* and to test whether the ETM, or derived models, adequately explain this variance. As mentioned above, previous work has established that both genetic and environmental factors are involved in the determination of aphally in *B. truncatus.* They have been more equivocal with regard to interaction between these factors, which may be due to the lack of genetic control. Assessing interactions indeed requires reaction norm experiments, that is, exposing genotypes to various environmental conditions (e.g., Lynch and Walsh 1998). However true genetic replicates (inbred lines) were not used in previous studies. Pure lines of *B. truncatus* were constituted and used in the present reaction norm experiment. Maternal effects were also checked. The ETM was refined by adding an error variance in the determination of individual environmental thresholds for each genotype separately. A maximum-likelihood procedure was derived to test both the significance of each term of models and their goodness of fit to observed reaction norms. This procedure was applied to aphally data, to determine which model should be used to predict the evolution of aphally. However, the maximum-likelihood procedure can be applied to any plastic binary trait.

Materials and Methods

Two experiments were performed. First, a reaction-norm experiment was conducted, involving 40 inbred lines from four populations. Parents were maintained at constant temperature, and their offspring reared at various temperatures. The offspring aphally ratio (AR) was determined and the influence of several parameters (e.g., inbred line or population) was analyzed. The ETM and derived models were tested using this dataset. Maternal effects (e.g., parental sexual morph) on AR at various temperatures were analyzed in a second experiment based on a more limited number of genotypes than the first one.

Organism and Rearing Conditions

Bulinus truncatus is a highly selfing snail species (Larambergue 1939; Viard et al. 1997). Sexual maturity of isolated individuals is gained in the laboratory at approximately 5 mm in length, that is, about 25 days of age above 25°C, but almost 75 days at 19°C (Doums et al. 1998a). Eggs are then layed continuously until death, which occurs in between four months and 12 months under laboratory conditions (Larambergue 1939). Aphallic individuals have been detected in all populations of *B. truncatus* so far studied, at frequencies generally higher than 50%. Phally status can easily be checked in snails larger than about 3 mm (see Jarne et al. 1992). More information on breeding biology and phally polymorphism in *B. truncatus* is given in Doums et al. (1998a).

The inbred lines used were set up from individuals collected in four natural populations from Niger (Boyze, Kobouri, Mari, and Namaga) in 1995. Their AR at sampling was 0.81, 0.00, 0.71, and 0.75, respectively (Doums et al. 1996b). These sites are separated by 100–450 km (Doums et al. 1996b; Viard et al. 1997). Each line was initiated from a single G_0 individual, and was maintained at 25°C under selfing, using the design described in Doums et al. (1996a). The individuals used were G_{10} for Boyze and Namaga, G_9 for Kobouri, and G_{14} for Mari, and were therefore considered as wholly homozygous. The number of lines available was 10 for Boyze, five for Kobouri, eight for Mari and 17 for Namaga. Over the whole experiment, snails were maintained under a 12:12 L:D photoperiod in 75-ml plastic boxes filled with water originating from the Lez spring (near Montpellier). Details on rearing temperatures are given below. Snails were fed ad libitum with boiled lettuce. Water and food were changed twice a week. The position of rearing boxes was randomly changed twice a week.

Reaction-Norm Experiment

Experimental design.—This experiment aimed at testing the effect of genetic and environmental factors on the AR. This was performed by estimating the AR at several temperatures among the offspring of individuals from inbred lines. One snail was randomly chosen before sexual maturity from each of the 40 lines, irrespective of its sexual morph. This provided seven aphallics (A) and three euphallics (E) in Boyze, five E in Kobouri, four A and four E in Mari, and 11 A and six E in Namaga. They were maintained isolated at 25°C. After sexual maturity, all capsules containing eggs were collected every two days over about six weeks (May 22 to June 19, 1998). The first batch of capsules was placed at 19°C, the second at 25°C, the third at 30°C, the fourth at 19°C, and so on up to the 14th batch (Fig. 1). After hatching, juveniles were maintained at the same temperature up to the determination of sexual morph. Note that full-sibs collected on the same day were reared in the same box. The position of the boxes in the rearing room was randomized twice a week. Phally status was determined in 2254 individuals (19 individuals, on average, for each of the 120 combinations of genotype and temperature).

Statistical analysis.—Because aphally is a binary trait, the relevant data for a given genotype is the probability of having a phallus at a given temperature. This was estimated as the AR among offspring within a line at a given temperature. The genetic factors included in the analysis were genotype (inbred line), parental sexual morph, and population of origin. The parental sexual morph was considered as a genetic factor. Indeed, because parental snails were chosen at random, their

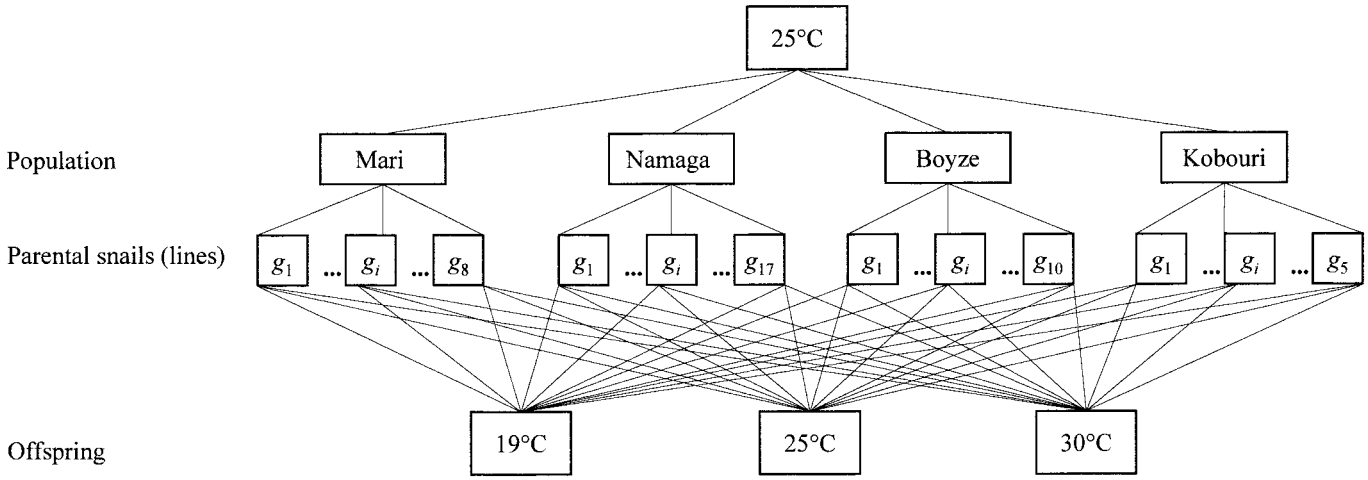

FIG. 1. Schematic diagram of the reaction-norm experiment. Inbred lines (g_i), which originated from four populations, were maintained at 25°C. Eggs were collected from each line and dispatched across three temperatures, and juveniles were subsequently maintained at the same temperature up to the determination of phally status.

morph can be assumed to give a rough estimate of their line AR at 25°C. The genotype factor was nested within the combination parental morph/population. The main environmental factor was the rearing temperature of offspring. Other environmental sources of variation were pooled into the error term of models. Data were analyzed using analyses of deviance under the framework of generalized linear models (Crawley 1993). The logistic-transformed probability of being aphallic a_{ijklm}, where i, j, k, l, and m stand for rearing temperature, population, parental sexual morph, genotype, and rearing box, respectively, was estimated as:

$$a_{ijklm} = \mu + t_i + p_j + m_k + tp_{ij} + tm_{ik} + pm_{jk} + g_{jkl} + tg_{ijkl} + tpm_{ijk} + b_{ijklm}, \quad (1)$$

where μ is the grand mean; t_i, p_j, and m_k refer to deviations from μ due to rearing temperature, population, and parental sexual morph respectively; g_{jkl} is the deviation due to genotype l from population j with parental sexual morph k; b_{ijklm} is the error term (or box factor); and other terms are interactions. The error term was assumed to follow a binomial distribution with parameter m (number of offspring within a box). The effects of factors and all possible interactions were tested through model reductions (Crawley 1993, ch. 12; Table 1). Overdispersion of data was accounted for following Crawley (1993, ch. 13). All calculations were performed using the GLIM package (Baker and Nelder 1985). Analyses were conducted using the whole dataset, as well as per temperature, per population, and per population/temperature combination.

We also computed broad-sense heritabilities of aphally for each temperature in each population and in the pooled dataset. Several methods have been developed to estimate the heri-

TABLE 1. Results of analyses of deviance in the reaction-norm experiment. The full model tested is given on the first line. μ is the grand mean; t_i, p_j, m_k, and g_{jkl} refer to offspring rearing temperature, population, parental sexual morph, and genotype (line), respectively; b_{ijklm} is the error term (box). Other terms are interactions. For each model, the term tested is in bold characters. Δdev (Δdf) is the change in residual deviance (degrees of freedom) between the model and the model minus the bold term. Test refers to the associated F-values (χ^2 for the full model), and P is the corresponding probability.

Model	Δdev	Δdf	Test	P
Error term				
$a_{ijklm} = \mu + t_i + p_j + m_k + tp_{ij} + tm_{ik} + pm_{jk} + g_{jkl} + tg_{ijkl} + tpm_{ijk} + \mathbf{b_{ijklm}}$	508.54	439	508.54	0.01
All G × E interactions				
$\mu + t_i + p_j + m_k + \mathbf{tp_{ij}} + \mathbf{tm_{ik}} + pm_{jk} + g_{jkl} + \mathbf{tg_{ijkl}} + \mathbf{tpm_{ijk}}$	196.18	78	2.17	$< 10^{-6}$
Specific G × E interactions				
$\mu + t_i + p_j + m_k + tp_{ij} + tm_{ik} + pm_{jk} + g_{jkl} + \mathbf{tg_{ijkl}} + tpm_{ijk}$	132.26	66	1.73	7×10^{-4}
$\mu + t_i + p_j + m_k + tp_{ij} + tm_{ik} + pm_{jk} + g_{jkl} + \mathbf{tpm_{ijk}}$	1.46	4	0.29	0.89
$\mu + t_i + p_j + m_k + tp_{ij} + \mathbf{tm_{ik}} + pm_{jk} + g_{jkl}$	18.84	2	7.47	6×10^{-4}
$\mu + t_i + p_j + m_k + \mathbf{tp_{ij}} + tm_{ik} + pm_{jk} + g_{jkl}$	25.37	6	3.35	0.003
All genetic terms				
$\mu + t_i + \mathbf{p_j} + \mathbf{m_k} + \mathbf{pm_{jk}} + \mathbf{g_{jkl}}$	921.18	39	17.31	$< 10^{-6}$
Specific genetic terms				
$\mu + t_i + p_j + m_k + pm_{jk} + \mathbf{g_{jkl}}$	436.68	33	9.71	$< 10^{-6}$
$\mu + t_i + p_j \pm m_k \pm \mathbf{pm_{jk}}$	15.91	2	3.83	0.02
$\mu + t_i + p_j + \mathbf{m_k}$	287.6	1	137.18	$< 10^{-6}$
$\mu + t_i + \mathbf{p_j} \pm m_k$	37.9	3	5.99	5×10^{-4}
Environmental term				
$\mu + \mathbf{t_i} + p_j + m_k + pm_{jk} \pm g_{jkl}$	137.12	2	50.30	$< 10^{-6}$

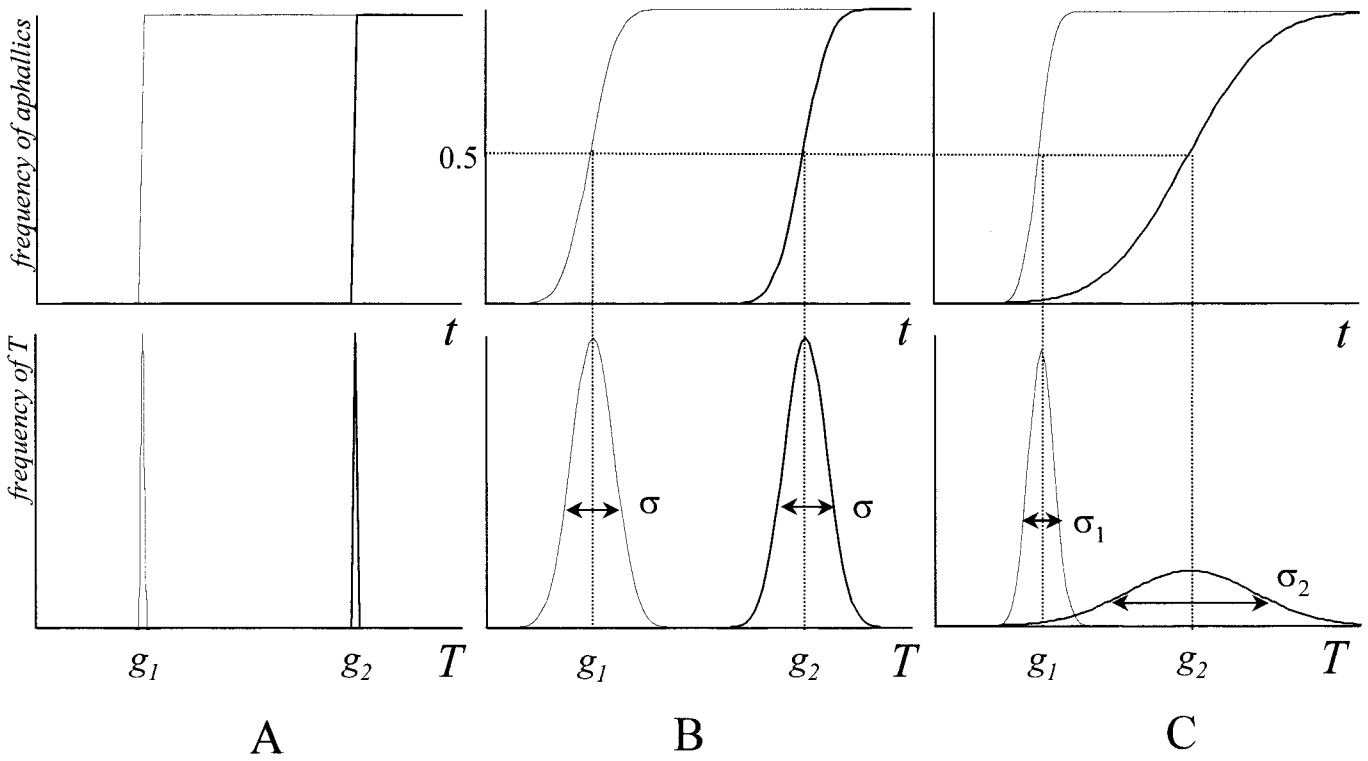

FIG. 2. Illustration of the environmental threshold model (modified from Roff 1994). The lower panels represent the distribution of *T*, the individual temperature threshold, among genotypes. Two genotypes (g_1 and g_2) are considered (light and heavy lines, respectively). The upper panels represent the predicted reaction norms for the same genotypes, that is, the alpha ratio as a function of temperature *t*. Three situations are pictured from left to rigth: (A) no microenvironmental variance ($\sigma_i = 0$); (B) constant microenvironmental variance ($\sigma_i > 0$); and (C) microenvironmental variance varying among genotypes ($\sigma_i > 0$). The genetically determined value G_i of *T* for genotype *i*, can be interpreted as the temperature at which the predicted aphally ratio is one-half.

tability of threshold characters, and they give similar results (Roff 1997; Lynch and Walsh 1998). The ANOVA method used is described ny Roff (1997, pp. 55–56) and estimates the heritability of the underlying trait (liability) determining the sexual morph under the classical TM. Briefly, data were encoded as 0 or 1 (for aphallics or euphallics, respectively) and analyzed as in a regular analysis of variance. The heritabilities on the 0/1 scale were then back-transformed into heritabilities on the underlying scale using formulae given in Roff (1997)

Test of the environmental threshold model.—The TM was used to estimate heritability at each temperature (hereafter referred to as macroenvironment, as opposed to microenvironment, which will be defined below). However, the TM does not include macroenvironmental effects. The reaction-norm data were therefore tested using the ETM and derived models (see introduction).

In the ETM, the continuous underlying trait is the individual threshold temperature, T_j (in temperature units; *j* refers to individuals). This threshold varies among individuals and behaves as a classical quantitative trait. In the case of aphally, individual *j* becomes euphallic if raised at a temperature below T_j and aphallic if raised in warmer conditions. If the microenvironmental variance, V_E, is negligible, the ETM assumes purely genetic effects. Therefore,

$$T_{ij} = g_i, \tag{2}$$

where *i* and *j* refer to individual *j* of genotype *i*, whose genetic value is g_i. In this form, the ETM takes the shape of a step function: at temperature *t*, individuals sharing the same genotype *i* should be either all euphallic ($t < g_i$) or all aphallic ($t > g_i$; see Fig. 2). This was obviously dismissed by our data, because intermediate ARs were found for most genotypes at each temperature (see Results). Therefore, a non-genetic component was incorporated, so that:

$$T_{ij} = g_i + e_{ij}, \tag{3}$$

where e_{ij} is normally distributed with mean zero and variance σ^2. σ^2 represents the usual microenvironmental variance, that is, the variation in T_{ij} due to developmental errors and/or uncontrolled environmental variation. As usually assumed in quantitative genetics models, σ^2 is constant and does not vary across genotypes (e.g., Lynch and Walsh 1998). When σ^2 differs from zero, intermediate AR may be observed for a given genotype at a given temperature. σ^2 controls the slope of the reaction norm: when σ^2 is zero, there is a sharp transition from 100% euphallic to 100% aphallic when temperature passes the threshold g_i. In contrast, the reaction norm is completely flat when σ^2 is infinite (Fig. 2). However, the shape of reaction norms may differ among genotypes. In the ETM framework, this means that genotypes vary in their ability to control developmental noise. This variation was not accounted for in the original version of the ETM (Hazel et

al. 1990). The adequate model in this case is still represented by equation (3), except that e_{ij} is now normally distributed with mean zero and variance σ_i^2 which varies among genotypes (Fig. 2).

Even when both g and σ^2 are allowed to vary among genotypes, the model may not perfectly fit the data. Indeed, the ETM assumes that the reaction norm of a given genotype takes the form of a cumulative normal distribution. For example, ARs must be either uniformly increasing or uniformly decreasing functions of temperature. With only two temperatures tested, one can always find an ETM (i.e., a value of [g, σ^2]) perfectly fitting the data. However, because our experiment was conducted at three temperatures, the extra degree of freedom can be used for testing whether the reaction norms significantly depart from the shape assumed by the ETM (i.e., cumulative normal). The ETM was therefore compared with a (full) model that fit separately ARs in each genotype and temperature.

The models described above are hierarchically related as follows: ETM_g (g estimated for each genotype, a single σ-value) is included into $ETM_{g\sigma}$ (a single value of both g and σ per genotype), which is itself included into the complete model (AR estimated for each genotype in each temperature). We also considered a null model ETM_0 assuming no genetic variation at all (a single value of both g and σ for all genotypes). The performances of the models were compared using likelihood-ratio tests. The log-likelihood of a model is:

$$\ln(L) = \sum_i \sum_t [a_{it}\ln(p_{it}) + e_{it}\ln(1 - p_{it})], \quad (4)$$

where a_{it} and e_{it} are the number of aphallic and euphallic individuals, respectively, produced by genotype i in temperature t and p_{it} is the corresponding probability of being aphallic under the model considered. For $ETM_{g\sigma}$:

$$p_{it} = \phi[(t - g_i)/\sigma_i], \quad (5)$$

where $\phi(x)$ is the surface of the right tail of a standard normal distribution down to x. The same formula applies for the ETM_g, replacing σ_i by σ and for ETM_0, replacing σ_i by σ and g_i by g. A Mathematica (Wolfram 1991) program (available upon request) was written for estimating numerically values of g and σ that maximize the likelihood under the $ETM_{g\sigma}$, ETM_g, and ETM_0. For the complete model, the best fit is obtained for

$$\hat{p}_{it} = \frac{a_{it}}{a_{it} + e_{it}}. \quad (6)$$

The maximum log-likelihood was obtained from equation (4). Models were compared by calculating the change in deviance $X^2 = 2\ 2ln(L)_{\text{model 1}} - ln(L)_{\text{model 2}})$. This follows a χ^2 distribution with $P_1 - P_2$ degrees of freedom (P_i being the number of independent parameters estimated in model i; see McCullagh and Nelder 1983).

Maternal-Effect Experiment

Experimental design.—In the previous experiment, the AR was estimated using a single parental snail per line. The genotype (line) effect could therefore be inflated by maternal effects, because parents were not replicated within lines. For example, aphallic and euphallic parents of identical genotype may produce different ARs among their offspring. This would compromise using parental phally status as a rough estimate of genetic tendency to aphally (as done in the reaction-norm experiment). This was evaluated using several aphallic and euphallic parents from a given line. In the previous experiment, parents always experienced a single temperature (25°C). Because the temperature experienced by parents may also affect their offspring AR, the experiment included different rearing temperatures for parents. The offspring AR was estimated at different temperatures. Because the number of combinations could quickly increase with such a design, we considered three genotypes and two temperatures only, that is, 24 combinations (three genotypes × two parental sexual morphs × two parental rearing temperatures × two offspring rearing temperatures). The experimental design is presented in Figure 3. The lines were chosen at random from the Namaga population. For each line, one box containing immature offspring from the previous experiment was chosen at random for each of two temperatures (25°C and 30°C). Offspring were isolated, and their sexual morph determined. One aphallic and one euphallic snail were chosen at random per genotype and temperature. These snails served as parents and were maintained at the same temperature over the whole experiment. Once all snails had reached sexual maturity, egg capsules were collected every three days. The first batch was placed at 25°C, the second at 30°C, the third at 25°C, and so on. Capsules were collected 12 times from July 1 to August 8, 1998. Juveniles hatching from these capsules were maintained at the same temperature and monitored until the determination of sexual morph (N = 700).

Statistical analysis.—Four factors were considered. Genotype (line) was the only genetic factor. The environmental factor was offspring rearing temperature, whereas the maternal effect factors were parental rearing temperature and parental sexual morph. The effect of factors and their interactions were tested using analyses of deviance (see above). The full model is presented below along with the corresponding results. Significance of terms was evaluated through model simplification.

RESULTS

Reaction-Norm Experiment

Analyses of deviance.—Results are presented in Table 1 and in Figures 4 and 5. We will consider successively error, interactions between genetic and environmental terms, and genetic and environmental terms. The error deviance (''box'' effect b_{ijklm}) of the full model was slightly larger than expected under binomial sampling (observed/expected = 508.54/439 = 1.16; first line in Table 1). However, significant overdispersion was restricted to Namaga at 25°C and 30°C (χ^2_{64} = 94.78, P = 0.007 at 25°C; χ^2_{67} = 89.12, P = 0.040 at 30°C).

Interactions between genetic and environmental factors (tg_{ijkl}, tpm_{ijk}, tp_{ij}, and tm_{ik}) were collectively highly significant (Table 1). This indicates genetic variation for the reaction norm of aphally to temperature, as illustrated in Figure 4. Individually, each of the four interaction terms was also highly significant, except tpm_{ijk} (Table 1). The significance of the

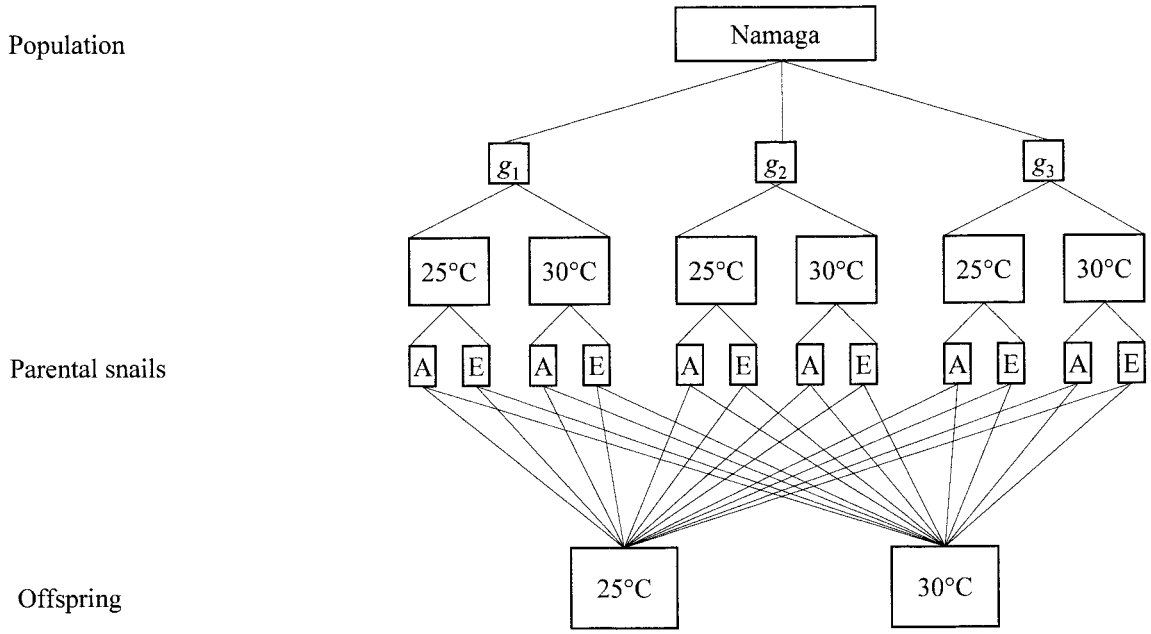

FIG. 3. Schematic diagram of the maternal-effect experiment. Parental snails were offspring from the reaction norm experiment. Individuals from three lines (Namaga) were raised at both 25°C and 30°C. For each line and and temperature, there was one aphallic and one euphallic snail. Their egg capsules were dispatched successively at 25°C and 30°C. Juveniles were maintained at the same temperature up to the determination of phally status.

tp_{ij} term indicates that part of the genetic variance for the reaction norm segregates among populations, whereas that of the tm_{ik} term indicates that the parental sexual morph is a reliable (if crude) indicator of this genetic variation. This clearly appears in Figure 4 when contrasting dashed and full lines. However, the population and parental sexual morph did not fully explain the genetic variation for reaction norms. Indeed, additional variation was due to genotypes within combinations of population and parental morph (term tg_{ijkl}). This is illustrated in Figure 4 by the marked variation in slopes among genotypes with either aphallic or euphallic parents.

Genetic factors (terms g_{jkl}, m_g, p_j, and pm_{jk}), when considered together, were highly significant (Table 1). These genetic differences could partly be accounted for by the population and parental sexual morph factors (p_j and m_k terms, respectively, in Table 1; Figs. 4, 5). However, as previously, there was significant variation among genotypes within combinations of population and parental morph (g_{jkl} term in Table 1; Fig. 4). The main effect of temperature was highly significant (t_i term in Table 1), and the AR increased with temperature (Figs. 4, 5).

Additional analyses were conducted within populations. A diversity of situations were observed (Fig. 4). In Namaga, Mari, and Boyze, genotype-by-environment interactions were detected ($tm_{ik} + tg_{ikl}$ term; in Mari: $F_{14,81} = 2.29$, $P = 0.011$; in Namaga: $F_{32,209} = 1.88$, $P = 0.005$; in Boyze: $F_{18,93} = 1.86$, $P = 0.029$). Genetic and environmental main effects were also significant in Mari and Namaga ($m_k + g_{kl}$ terms in Mari: $F_{7,95} = 22.29$, $P < 10^{-6}$; in Namaga: $F_{16,241} = 17.80$, $P < 10^{-6}$; term in Mari: $F_{2,95} = 13.91$, $P < 10^{-4}$; in Namaga: $F_{2,241} = 20.10$, $P < 10^{-6}$). In contrast, Boyze genotypes were on average insensitive to temperature (t_i term: $F_{2,111} = 0.30$, $P = 0.741$), although they displayed markedly different ARs ($m_k + g_{kl}$ terms: $F_{9,111} = 12.33$, $P < 10^{-6}$). The opposite situation was found in Kobouri, in which genotypes were sensitive to temperature (t_i term: $F_{2,64} = 38.62$, $P < 10^{-6}$), but did not differ in mean ARs (g_l term: $F_{4,64} = 1.72$, $P = 0.16$; note that m_k was not included in the analysis because all parents were euphallic) or reaction norm to temperature (tg_{il} term: $F_{8,56} = 1.03$, $P = 0.43$). Analyses were also conducted within temperatures and within the 12 population-temperature combinations. Genetic variation was very significant at 19°C and 25°C among and within populations (data not shown); however, little variation was detected at 30°C, with no significant population effect and no significant genotype effect within populations, except for Namaga. The AR was indeed high and similar in all populations (Fig. 5). Note that differential mortality could not introduce detectable differences in AR among genotypes, because mortality was overall very low (<10%).

Estimating broad-sense heritability.—The results were consistent with those obtained from the analyses of deviance. First, high values of broad-sense heritability were detected when populations were pooled, and heritability decreased with increasing temperature (Table 2). Second, all populations had large heritabilities, except Kobouri (Table 2).

Test of the environmental threshold model.—Results are presented in Table 3. ETM_g (genetic variation on g defining the threshold T_{ij} and fixed developmental noise χ) performed better than ETM_0 (fixed g and σ), but was outperformed by $ETM_{g\sigma}$ (variation on both g and σ). There was significant residual variance, because $ETM_{g\sigma}$ was itself outperformed by the complete model. In other words, the reaction norms differed significantly from cumulative normal distribution.

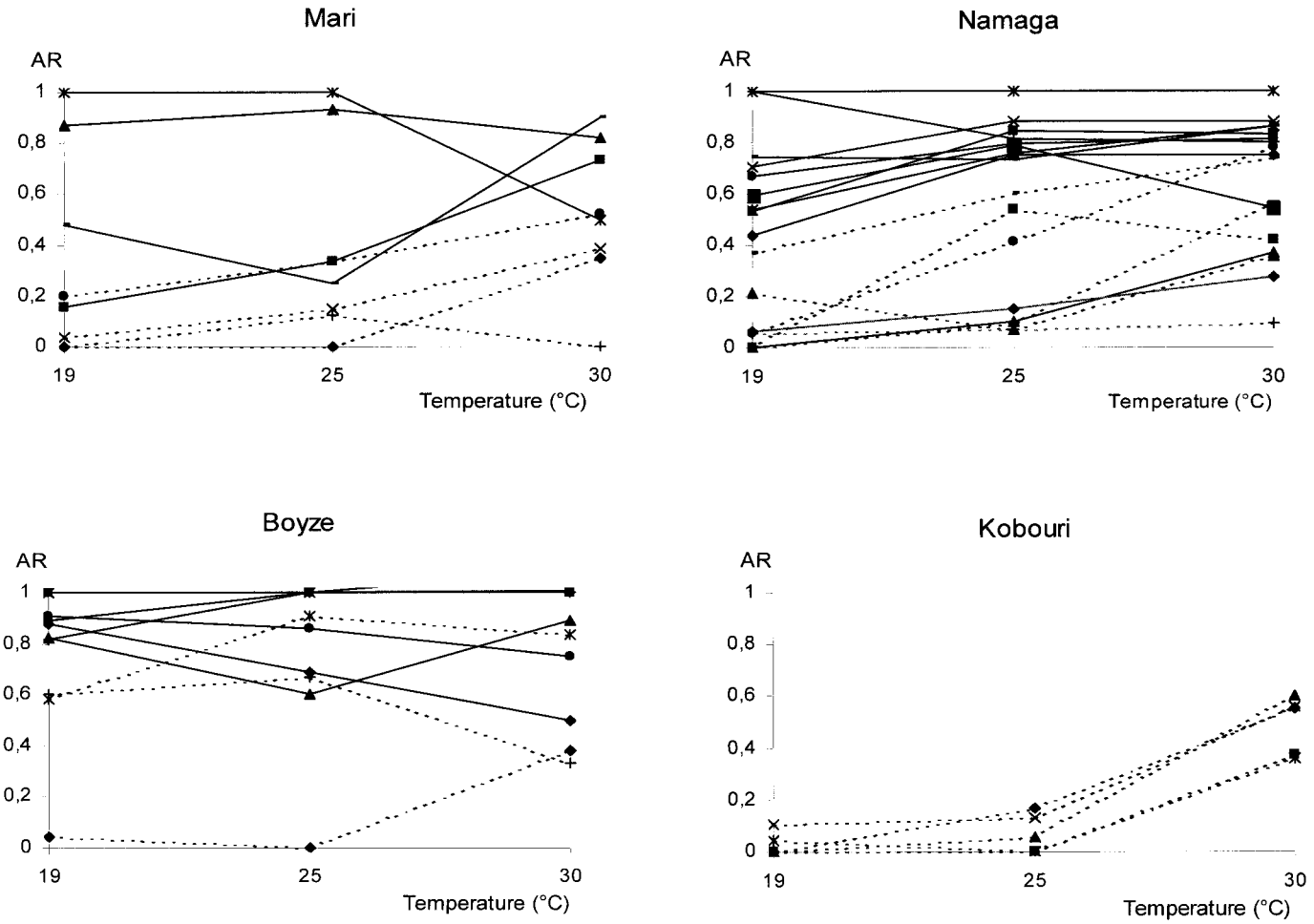

FIG. 4. Reaction norms of aphally ratio (AR) to temperature within four populations. Each line represents a genotype. Dashed and solid lines refers to genotypes for which euphallic and aphallic parents were used, respectively.

Maternal-Effect Experiment

The models tested and associated results are given in Table 4. The error variance was slightly larger than expected under binomial sampling (observed/expected = 80.41/52 = 1.55; first line in Table 4). The detailed analysis per genotype showed that this was due to a single genotype (error term for g_3:χ^2_{18} = 41.22, P = 0.001). None of the 11 interaction terms were significant (Table 4). r_j (parental rearing temperature) and m_k (parental sexual morph) were considered as maternal effects. Note that the parental morph effect was not considered as a genetic effect as in the reaction-norm experiment because parental morphs were compared within genotypes. The first one was highly significant, whereas the second one was not. Significant genetic variation was also detected (g_l term). In contrast, t_i (offspring rearing temperature) was not significant. Further analyses indicated that the influence of parental rearing temperature was significant for g_2 only ($F_{1,22}$ = 23.25, $P = 8 \times 10^{-5}$). The parental sexual morph factor was never significant within genotypes (g_1: $F_{1,22}$ = 0.00, P = 0.96; g_2: $F_{1,22}$ = 1.87, P = 0.19; g_3: $F_{1,23}$ = 0.03, P = 0.87). We also tested for maternal effects at a given temperature by adding all maternal effects other than parental rearing temperature ($rm_{jk} + rmg_{jkl} + rg_{jl} + m_k + mg_{kl}$) to the minimal model (including only significant terms, that is, and g_l) . This was not significant ($F_{8,64}$ = 1.84, P = 0.09), indicating that individuals from the same line reared at the same temperature produce offspring with similar AR.

DISCUSSION

In this study, we attempted to identify the source of phenotypic variation for a binary trait, aphally. We focused on the respective roles of genetic and environmental factors, as well as on their interaction, and the validity of the ETM. These two aspects will be discussed in turn, highlighting the assessment of genotype-by-environment interactions and the test of ETM-like models.

The results of the reaction-norm experiment (hereafter RNE) indicate that a significant proportion of phenotypic variation for the sexual morph in *B. truncatus* is of genetic nature. Indeed, high broad-sense heritability values were obtained in most populations as well as in the pooled sample (up to 0.9). This is consistent with previous results obtained by Doums et al. (1996a) in the same populations. These values were 0.479 (SE = 0.066) and 0.756 (0.116) at 25°C for Namaga and Mari, respectively, which are comparable to the 0.559 (0.144) and 0.790 (0.270) values obtained here. In

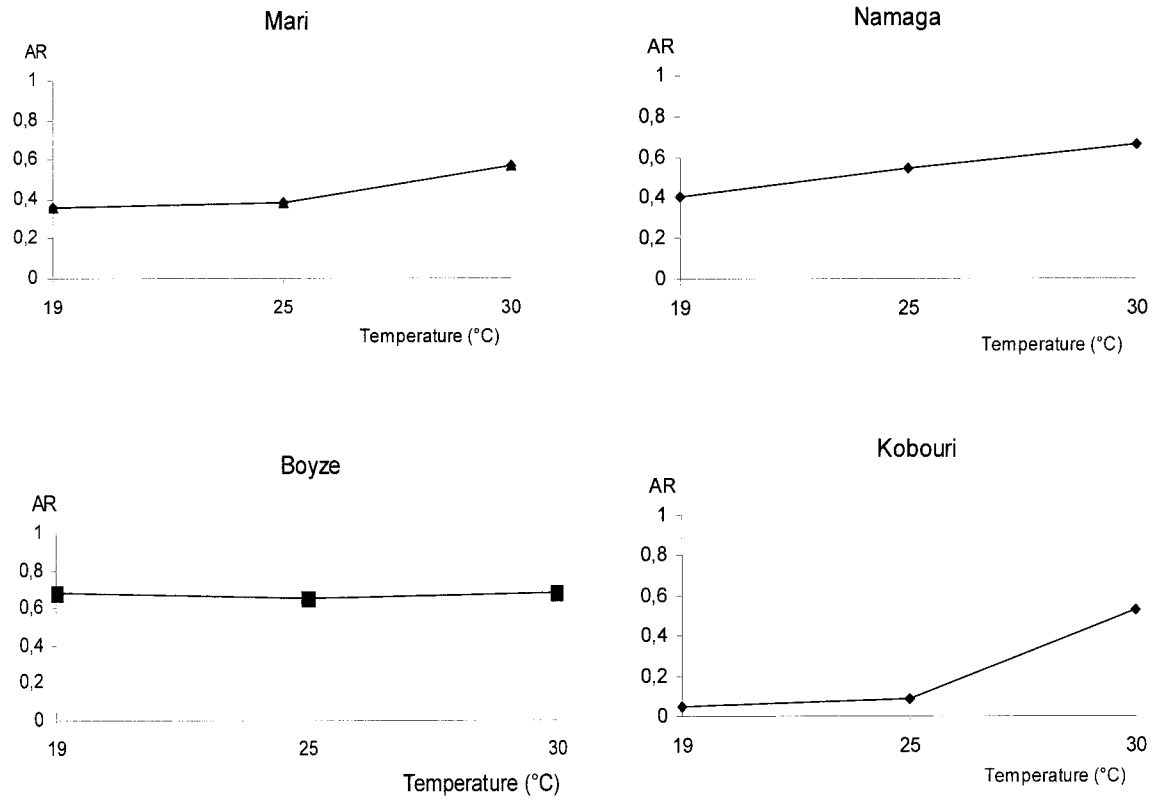

FIG. 5. Reaction norms of aphally ratio (AR) to temperature within each population. The AR was estimated as the proportion of aphallic offspring over all lines at each temperature.

contrast, Schrag et al. (1992) found no heritability, probably because they used genetically similar snails. We also observed that the genetic variation is distributed among individuals within populations, in agreement with previous data (Doums et al. 1996a,b).

Genetic effects may be upwardly biased. Uncontrolled environmental variation at the box level could indeed create artificial resemblance among boxmates, because several offspring of the same line were raised in the same box. However, several boxes (typically four to five) were set up and their position was regularly randomized for each line and temperature (largely averaging out potential box effects), and only limited variation was detected among boxes within lines and temperatures (low overdispersion). This certainly reduces the bias due to shared environment. Maternal effects, especially the parental sexual morph, may constitute a further source of bias. The results of the RNE do show that lines represented by aphallic parents yielded highly aphallic offspring (for technical reasons, it was impossible to standardize the parental sexual morph over lines). However, the maternal-effect experiment (hereafter MEE) performed on a restricted number of lines, indicated that the influence of the parental morph was not significant within lines and temperatures. The parental morph in the RNE is therefore not a causal determinant of the offspring AR, but rather a correlate of common genetic factors. In other words, the parental sexual morph behaves as a rough indicator of genetically determined AR, not as a source of nongenetic variation. This is in line with previous studies (Schrag and Read 1992; Schrag et al. 1992; Doums et al. 1996a). The MEE design allowed to check for another maternal effect, the parental rearing temperature. A significant effect was detected for one genotype only (of three). This result is puzzling because no effect of the offspring temperature was detected. Indeed, the three randomly chosen genotypes happened to show a rather flat response to temperature in the RNE experiment. The parental temperature

TABLE 2. Broad-sense heritability (on the underlying scale) estimated at three temperatures either for the whole dataset or for each population separately. SE and sample size are given in parentheses.

	Temperature		
	19°C	25°C	30°C
All data	0.79 (0.099, 785)	0.71 (0.096, 800)	0.27 (0.068, 669)
Boyze	0.59 (0.253, 125)	0.50 (0.224, 111)	0.45 (0.280, 92)
Kobouri	0.09 (0.283, 102)	−0.07 (0.067, 131)	−0.108 (0.023, 111)
Mari	0.92 (0.257, 173)	0.79 (0.270, 144)	0.24 (0.185, 121)
Namaga	0.82 (0.156, 385)	0.56 (0.144, 414)	0.35 (0.124, 345)

TABLE 3. Results of the likelihood-ratio tests performed on the ETMs. ln(L) is the log-likelihood and Dev. the total deviance (i.e., twice the difference in ln[L]) between the model and the complete model) of the corresponding model. P_i refers to the number of free parameters, χ^2 to twice the difference in log-likelihood between two successive models, $P_i - P_j$ to the difference in number of free parameters between two successive models, and P to the corresponding probability of the χ^2 test.

Model	ln(L)	Dev.	P_i	χ^2	$P_i - P_j$	P
Complete	956.89	0	120	complete vs. $ETM_{g\sigma}$		
				74.89	40	7×10^{-4}
$ETM_{g\sigma}$	994.33	74.89	80	$ETM_{g\sigma}$ vs. ETM_g		
				131.59	39	5×10^{-12}
ETM_g	1060.13	206.48	41	ETM_g vs. ETM_0		
				959.93	39	5×10^{-176}
ETM_0	1515.59	1166.41	2			

effect and the lack of offspring temperature effect in the MEE is possibly due to the fact that eggs experienced the parental rearing temperature before their transfer, although for a limited time only. The effect of offspring temperature is therefore partly confounded with the effect of parental temperature in the MEE. This is not the case in the RNE, where only one parental temperature was used (25°C). Despite this puzzling result, the maternal effect due to parental temperature, if present, does not inflate the variation among genotypes detected in the RNE because the parental temperature was 25°C for all genotypes. On the whole, the MEE does not dismiss maternal effects in a clear-cut manner. However, it provides some evidence to the hypothesis that most of the variation observed in the RNE is of genetic nature.

The RNE confirms the influence of temperature on AR (Schrag and Read 1992; Doums et al. 1996a) and, more interestingly, indicates a significant genotype-by-environment interaction. Doums et al. (1996a), using families of G_3 full-sibs from Mari, failed to detect such an interaction in a similar reaction norm experiment. Because the snails they used were the ancestors of the snails used here, their result probably derives from the lack of true genetic replicates. Three generations of selfing (as in Doums et al. 1996a) may not permit elimination of the within-family genetic variance for aphally, making the detection of interaction difficult. Genotype-by-environment interactions had been analyzed for other binary traits. For example, Roff (1994) found no interaction for the proportion of macropterous individuals in three species of crickets, when the environments differed by a single variable (temperature or photoperiod). However, Rhen and Lang (1998), investigating sex ratio in reptiles, found that family interacted significantly with temperature in two of three species studied, although no interaction was found by Janzen (1992) in one of these species. (Note though that the reaction norm of sex ratio in reptiles is not a monotonic function of temperature, making a comparison with aphally rather hazardous.) Genotype-by-environment interactions are of significant importance for the evolution of binary traits because genetic variation for reaction norms allows selection to act on phenotypic plasticity. This certainly highlights the necessity of developing new models for the evolution of sexual polymorphisms, since the role and evolution of sexual plasticity has not been accounted for in previous models (e.g., for gynodioecy, Gouyon et al. 1991; for aphally, Doums et al. 1998b). Population structure must also be taken into account, as our results indicate that the populations of *B. truncatus* exhibit very different potentials for the evolution of sexual plasticity. Genetic variation for reaction norms was found in all populations but Kobouri. Kobouri lines are sensitive to temperature, but do not show genetic variation for the AR at any temperature. In contrast, lines from Boyze were genetically variable, although on average not sensitive to temperature.

Studying the evolution of reaction norms requires appropriate descriptive models. Hazel et al. (1990), addressing the question of ''how selection acts to mould alternative phenotypes (or strategies) to evolutionary optima,'' developed the ETM for binary characters. They considered some microenvironmental variation among individuals around the genetically determined threshold, though did not include it as a parameter in the model (see Fig. 2A, B). We extended the ETM to allow for both nongenetic variance for thresholds and variation of this variance among genotypes. These ETM-derived models were evaluated using a framework based on maximum-likelihood procedures. First, our results cannot be explained by an ETM that assumes purely genetic effects. The AR of a given genotype at constant temperature is indeed generally intermediate, while such an ETM predicts it to be either zero, or one (Fig. 2A). Adding microenvironmental variance is therefore required, as previously hypothesized by Roff (1994) and Doums et al. (1996a). However, these authors were not able to test their hypothesis, due to inappropriate experimental designs—they could not separate within-line genetic variance from microenvironmental variance. Here the improved ETM (ETM_g in Table 3) explains 82% of the deviance when compared to the null model. However, an even more detailed version, including genotype-specific microenvironmental variance ($ETM_{g\sigma}$) yielded an additional 11% decrease in deviance when compared to ETM_g. Because heterozygosity has often been related to developmental stability (Lerner 1954; Eanes 1978; Mitton 1978; Deng 1997), one may wonder whether this result is an artefact due to the extreme homozygosity following enforced selfing in the lines used. However, heterozygosity-stability relationships have been established in naturally outcrossing species and considered as an expression of inbreeding depression. In contrast, natural populations of *B. truncatus* have an average selfing rate above 80%, and no inbreeding depression has been detected to date (Doums et al. 1998b). Moreover, repeated enforced selfing allowed here to standardize inbreeding levels, which naturally vary among individuals directly taken from the field.

The variation in developmental noise (σ_i) among genotypes is better interpreted as the expression of genotype-by-envi-

TABLE 4. Results of analyses of deviance in the maternal effect experiment. The full model tested is given on the first line. μ is the grand mean; t_i, r_j, m_k, and g_l refer to offspring rearing temperature, parental rearing temperature, parental sexual morph, and genotype (line), respectively; b_{ijklm} is the error term (box effect). Other terms are interactions. See legend of Table 1.

Model	Δdev	Δdf	Test	P
$\mu + t_i + r_j + m_k + g_l + tr_{ij} + tm_{ik} + rm_{jk} + tg_{il} + rg_{jl} + mg_{kl} + trm_{ijk} + trg_{ijl} + tmg_{ikl} + rmg_{jkl} + \boldsymbol{b_{ijklm}}$	80.41	52	80.41	0.007
$\mu + t_i + r_j + m_k + g_l + tr_{ij} + tm_{ik} + rm_{jk} + tg_{il} + rg_{jl} + mg_{kl} + trm_{ijk} + trg_{ijl} + tmg_{ikl} + rmg_{jkl} + \boldsymbol{trmg_{ijkl}}$	3.91	2	1.27	0.30
$\mu + t_i + r_j + m_k + g_l + tr_{ij} + tm_{ik} + rm_{jk} + tg_{il} + rg_{jl} + mg_{kl} + trm_{ijk} + trg_{ijl} + tmg_{ikl} + \boldsymbol{rmg_{jkl}}$	5.95	2	1.90	0.15
$\mu + t_i + r_j + m_k + g_l + tr_{ij} + tm_{ik} + rm_{jk} + tg_{il} + rg_{jl} + mg_{kl} + trm_{ijk} + trg_{ijl} + \boldsymbol{tmg_{ikl}} + rmg_{jkl}$	1.06	2	0.34	0.71
$\mu + t_i + r_j + m_k + g_l + tr_{ij} + tm_{ik} + rm_{jk} + tg_{il} + rg_{jl} + mg_{kl} + trm_{ijk} + \boldsymbol{trg_{ijl}} + \boldsymbol{tmg_{ikl}} + rmg_{jkl}$	0.68	2	0.22	0.81
$\mu + t_i + r_j + m_k + g_l + tr_{ij} + tm_{ik} + rm_{jk} + tg_{il} + rg_{jl} + mg_{kl} + \boldsymbol{trm_{ijk}} + trg_{ijl} + tmg_{ikl} + rmg_{jkl}$	0.36	1	0.23	0.16
$\mu + t_i + r_j + m_k + g_l + tr_{ij} + tm_{ik} + rm_{jk} + tg_{il} + rg_{jl} + \boldsymbol{mg_{kl}} + trm_{ijk} + trg_{ijl} + tmg_{ikl} + rmg_{jkl}$	1.96	2	0.65	0.52
$\mu + t_i + r_j + m_k + g_l + tr_{ij} + tm_{ik} + rm_{jk} + tg_{il} + \boldsymbol{rg_{jl}} + mg_{kl}$	8.98	2	2.99	0.06
$\mu + t_i + r_j + m_k + g_l + tr_{ij} + tm_{ik} + rm_{jk} + \boldsymbol{tg_{il}} + rg_{jl} + mg_{kl}$	0.84	2	0.28	0.76
$\mu + t_i + r_j + m_k + g_l + tr_{ij} + tm_{ik} + \boldsymbol{rm_{jk}} + tg_{il} + rg_{jl} + mg_{kl}$	1.17	1	0.78	0.38
$\mu + t_i + r_j + m_k + g_l + tr_{ij} + \boldsymbol{tm_{ik}} + rm_{jk} + tg_{il} + rg_{jl} + mg_{kl}$	0.25	1	0.16	0.69
$\mu + t_i + r_j + m_k + g_l + \boldsymbol{tr_{ij}} + tm_{ik} + rm_{jk} + tg_{il} + rg_{jl} + mg_{kl}$	1.07	1	0.71	0.40
$\mu + t_i + r_j + m_k + \boldsymbol{g_l} + tr_{ij} + tm_{ik} + rm_{jk} + tg_{il} + rg_{jl} + mg_{kl}$	32.80	2	10.72	9×10^{-5}
$\mu + t_i + r_j + \boldsymbol{m_k} + g_l + tr_{ij} + tm_{ik} + rm_{jk} + tg_{il} + rg_{jl} + mg_{kl}$	1.49	1	0.97	0.32
$\mu + t_i + \boldsymbol{r_j} + m_k + g_l + tr_{ij} + tm_{ik} + rm_{jk} + tg_{il} + rg_{jl} + mg_{kl}$	28.99	1	18.95	4×10^{-5}
$\mu + \boldsymbol{t_i} + r_j + m_k + g_l + tr_{ij} + tm_{ik} + rm_{jk} + tg_{il} + rg_{jl} + mg_{kl}$	0.03	1	0.02	0.89

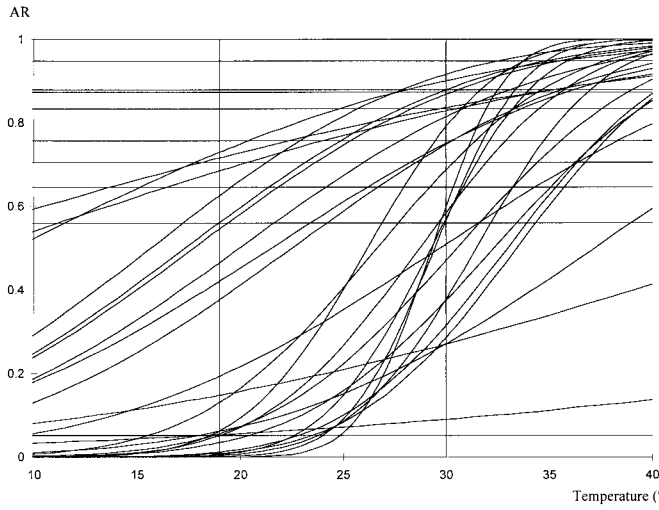

FIG. 6. Reaction norms of aphally ratio (AR) to temperature represented as cumulative normal distributions obtained using the $ETM_{g\sigma}$ (see text for more details). Each line represents one genotype, and the 40 lines are shown.

ronment interaction (Fig. 6), since σ_i controls the slope of reaction norms (Figs. 2, 6). As a consequence, the basis of aphally is better described using the $ETM_{g\sigma}$ than the ETM_g, that is, when considering genotype-specific developmental noise. Note that other research fields in evolutionary biology include such a noise as an important parameter (Gavrilets and Hastings 1994), for example the theory of developmental homeostasis (Lerner 1954). Empirical studies provide some ground for such a position (e.g., Deng 1997). However, $ETM_{g\sigma}$ does not fit perfectly the present data, leaving a small (6%) but significant residual deviance when compared to the complete model. This means that the reaction norms do not exactly take the shape assumed by ETMs—a cumulative normal function. It remains that the ETM has too much power, both qualitative and quantitative, to be rejected (at least the $ETM_{g\sigma}$ version) when describing aphally.

More generally, the maximum-likelihood framework used here can be applied to any plastic binary trait, assuming that experimental data, especially on genotype-by-environment interactions, are available. There are at least two advantages associated with the approach based on ETMs. First reaction norms are modeled in terms of developmental noise (σ_i). Whether this has any biological sense or should be considered as a purely phenomenological way of describing reaction norms is a matter of interpretation. However, developmental noise is a central component of evolutionary models (Lerner 1954) and its relationships with phenotypic plasticity have been underlined. For example, the canalization concept includes both restricted developmental noise and insensitivity to macroenvironmental variation (Waddington 1942; Zakharov 1992). In this context, the ETM approach has the advantage of providing a clear, mathematically explicit relationship between these two sources of variation.

Second it is very convenient to describe reaction norms using a few parameters only. This is not without analogy with the polynomial models often used for continuous traits (Via et al. 1995). Modeling the evolution of binary traits

indeed requires a thorough description of genotype-by-environment interactions, as the latter allow not only for selection on plasticity, but also for the maintenance of variability in natural populations subject to macroenvironmental variation.

With regard to aphally, the adaptive nature of plasticity remains unknown because the selective forces involved have not been characterized in natural populations. This situation parallels that of environmental sex determination, for which adaptive hypotheses received ambiguous support from empirical data (Bull and Charnov 1989; Rhen and Lang 1998). In the current models, the evolution of aphally depends on natural selection on selfing rates, that is, inbreeding depression, the cost of the male function, and the shape of the relationship between the selfing rate and AR (Doums et al. 1998b). Because the range of values for these parameters in *B. truncatus* is not yet well assessed, the relevant selective pressures acting on ARs are still uncertain. Sensitivity of the sexual morph to temperature has to be added to this list of parameters. Indeed, temperature may correlate with conditions under which selfing is selected. Schrag et al. (1994a) detected a correlation between AR and parasitic prevalence in Nigerian populations of *B. truncatus* and proposed that euphallic individuals would be favored at low temperatures if the parasitic pressure increases when temperature decreases. This hypothesis assumes that outcrossed progeny have a better fitness than selfed progeny in the presence of parasites, as suggested by the Red Queen hypothesis (Bell 1982). However, many parameters other than parasitic pressure, such as population density or metabolic maintenance costs, may correlate with temperature and modify the selective pressure on aphally. These hypotheses remain ad hoc speculations in the absence of relevant data. However, because *Bulinus* populations are unstable and frequently experience bottlenecks (Brown 1994; Vera et al. 1994; Viard et al. 1997), short-term patterns at the scale of one or a few natural populations may reflect recent random events rather than equilibrium situations optimized by natural selection.

Acknowledgments

We thank C. Doums, F. Justy, M.-A. Perdieu, and F. Viard, whose help was invaluable for maintaining the snail lines; C. Doums for discussions; and C. Doums and two anonymous reviewers for comments on the manuscript. This work was supported by funds from the Centre National de la Recherche Scientifique (CNRS) to UMR 5554 (Institut des Sciences de l'Evolution) and UPR 9056 (Centre d'Ecologie Fonctionnelle et Evolutive). This work is part of the Ph.D. project of M-FO, which is supported by funds from the Natural Sciences and Engineering Research Council of Canada (NSERC).

Literature Cited

Baker, R. J., and J. A. Nelder. 1985. The GLIM system. Rel. 3.77, manual. Numerical Algorithms Group, Oxford, U.K.

Barrett, S. C. H. 1994. The evolutionary biology of tristyly. Oxf. Surv. Evol. Biol. 9:283–326.

Baur, B., X. Chen, and A. Baur. 1993. Genital dimorphism in natural populations of the land snail *Chondrina clienta* and the influence of the environment on its expression. J. Zool., Lond. 231: 275–284.

Bell, G. 1982. The masterpiece of nature: the evolution and genetics of sexuality. Univ. of California Press, Berkeley.

Brown, D. S. 1994. Freshwater snails of Africa and their medical importance. Taylor and Francis Ltd., London.

Bull, J. J., and E. L. Charnov. 1989. Enigmatic reptilian sex ratios. Evolution 43:1561–1566.

Couvet, D., A. Atlan, E. Belhassen, C. Gliddon, P.-H. Gouyon, and F. Kjellberg. 1990. Co-evolution between two symbionts: the case of cytoplasmic male-sterility in higher plants. Oxf. Surv. Evol. Biol. 7:225–249.

Crawley, M. J. 1993. GLIM for ecologists. Blackwell Scientific Publications, Oxford, U.K.

Darwin, C. R. 1877. The different forms of flower on plants of the same species. Murray, London.

Deng, H.-W. 1997. Increase in developmental instability upon inbreeding in *Daphnia.* Heredity 78:182–189.

Doums, C., and P. Jarne. 1996. The evolution of phally polymorphism in *Bulinus truncatus* (Gastropoda, Planorbidae): the cost of male function analysed through life-history traits and sex allocation. Oecologia 106:464–469.

Doums, C., P. Brémond, B. Delay, and P. Jarne. 1996a. The genetical and environmental determination of phally polymorphism in the freshwater snail *Bulinus truncatus.* Genetics 142: 217–225.

Doums, C., R. Labbo, and P. Jarne. 1996b. Stability and genetical basis of variability of phally polymorphism in natural populations of the self-fertile freshwater snail *Bulinus truncatus.* Genet. Res. Camb. 68:22–33.

Doums, C., M.-A. Perdieu, and P. Jarne. 1998a. Resource allocation and stressful conditions in the aphallic snail *Bulinus truncatus.* Ecology 79:720–733.

Doums, C., F. Viard, and P. Jarne. 1998b. The evolution of phally polymorphism. Biol. J. Linn. Soc. 64:273–296.

Eanes, W. F. 1978. Morphological variance and enzyme heterozygosity in the monarch butterfly. Nature 276:263–264.

Falconer, D., S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. Longman.

Gavrilets, S., and A. Hastings. 1994. A quantitative model for selection on developmental noise. Evolution 48:1478–1486.

Gouyon, P. H., F. Vichot, and J. M. M. Van Damme. 1991. Nuclear-cytoplasmic male sterility: single-point equilibria versus limit cycles. Am. Nat. 137:498–514.

Hazel, W. N., R. Smock, and M. D. Johnson. 1990. A polygenic model for the evolution and maintenance of conditional strategies. Proc. R. Soc. Lond B Biol. Sci. 242:181–187.

Janzen, F. J. 1992. Heritable variation for sex ratio under environmental sex determination in the common snapping turtle (*Chelydra serpentina*). Genetics 131:155–161.

Janzen, F. J., and G. L. Paukstis. 1991. Environmental sex determination in reptiles: ecology, evolution, and experimental design. Q. Rev. Biol. 66:149–179.

Jarne, P., L. Finot, C. Bellec, and B. Delay. 1992. Aphally versus euphally in self-fertile hermaphrodite snails from the species *Bulinus truncatus* (Pulmonata: Planorbidae). Am. Nat. 139: 424–432.

Larambergue, M. de 1939. Etude de l'autofécondation chez les gastéropodes pulmonés: recherches sur l'aphallie et la fécondation chez *Bulinus* (*Isidora*) *contortus* Michaud. Bull. Biol. Fr. Belg. 73:19–231.

Lerner, I. M. 1954. Genetic homeostasis. Oliver and Boyd, London.

Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer, Sunderland, MA.

McCullagh, H. P., and J. A. Nelder. 1983. Generalized linear models. Cambridge Univ. Press, Cambridge.

Mitton, J. B. 1978. Relationship between heterozygosity forenzyme loci and variation of morphological characters in natural populations. Nature 273:661–662.

Paoletti, C., and K. E. Holsinger. 1999. Spatial patterns of polygenic variation in *Impatiens capensis,* a species with an environmental controlled mixted mating system. J. Evol. Biol. 12:689–696.

Rhen, T., and J. W. Lang. 1998. Among-family variation for environmental sex determination in reptiles. Evolution 52: 1514–1520.

Roff, D. A. 1986. The genetic basis of wing dimorphism in the sand cricket, *Gryllus firmus* and its relevance to the evolution of wing dimorphisms in insects. Heredity 57:221–231.

———. 1994. The evolution of dimorphic traits: predicting the genetic correlation between environments. Genetics 136: 395–401.

———. 1996. The evolution of threshold traits in animals. Q. Rev. Biol. 71:3–34.

———. 1997. Evolutionary quantitative genetics. Chapman and Hall, London.

Ronfort, J., and D. Couvet. 1995. A stochastic model of selection on selfing rates in structured populations. Genet. Res. Camb. 65: 209–222.

Sassaman, C. 1989. Inbreeding and sex ratio variation in female-biased populations of a clam shrimp, *Eulimnadia texana.* Bull. Mar. Sc. 45:425–432.

Schrag, S. J., and A. F. Read. 1992. Temperature determination of male outcrossing ability in a simultaneous hermaphrodite. Evolution 46:1698–1707.

Schrag, S. J., D. Rollinson, A. E. Keymer, and A. F. Read. 1992. Heritability of male outcrossing ability in the simultaneous hermaphrodite, *Bulinus truncatus* (Gastropoda: Planorbidae). J. Zool. Lond. 226:311–319.

Schrag, S. J., A. O. Mooers, T. N'Difon, and A. F. Read. 1994a. Ecological correlates of male outcrossing ability in a simultaneous hermaphrodite snail. Am. Nat. 143:636–655.

Schrag, S. J., G. T. Ndifon, and A. F. Read. 1994b. Temperature-determined outcrossing ability in wild populations of a simultaneous hermaphrodite snail. Ecology 75:2066–2077.

Van Damme, J. M. M. 1983. Gynodioecy in *Plantago lanceolata* L. II Inheritance of three male sterility types. Heredity 50: 253–273.

Vera, C., P. Brémond, R. Labbo, F. Mouchet, E. Sellin, D. Boulanger, J.-P. Pointier, B. Delay, and B. Sellin. 1994. Seasonal fluctuations in population densities of *Bulinus senegalensis* and *B. truncatus* (Planorbidae) in temporary pools in a focus of *Schistosoma haematobium* in Niger: implications for control. J. Moll. Stud. 61:79–88.

Via, S., R. Gomulkiewicz, G. de Jong, S. M. Scheiner, C. D. Schlichting, P. H. Van Tienderen. 1995. Adaptive phenotypic plasticity: consensus and controversy. Trends Ecol. Evol. 10: 212–217.

Viard, F., C. Doums, and P. Jarne. 1997. Selfing, sexual polymorphism and microsatellites in the hermaphroditic freshwater snail *Bulinus truncatus.* Proc. R. Soc. Lond. B Biol. Sci. 264:39–44.

Waddington, C. H. 1942. Canalization of development and the inheritance of acquired characters. Nature 150:563–565.

Waller, D. M. 1980. Environmental determinants of outcrossing in *Impatiens capensis* (Balsaminaceae). Evolution 34:747–761.

Wolfram, S. 1991. Mathematica: a system for doing mathematics by computer. Addison-Wesley, Paris.

Zakharov, V. M. 1992. Population phenogenetics: analysis of developmental stability in natural populations. Acta Zool. Fenn. 191:7–130.

Corresponding Editor: S. Edwards